

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Needleman et al.)
Serial No.: 08/785,997) Attorney Docket
Filed: January 21, 1997) 6221/68346
For: AN IMMUNOLOGICAL PROCESS) MON-101.0
FOR INCREASING THE HDL)
CHOLESTEROL CONCENTRATION)
Examiner: T. Scheiner) Art Group:
) 1642

Considered
4E
9/10/97

DECLARATION OF PHILIP NEEDLEMAN AND
KEVIN GLENN PURSUANT TO 37 C.F.R. §1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PHILIP NEEDLEMAN, Ph.D. and KEVIN GLENN, Ph.D.

Declare:

1. That they are the Philip Needleman and Kevin Glenn that are the named inventors of the subject patent application;
2. That they are employed by G.D. Searle, Co., (Searle) a wholly owned subsidiary of the Monsanto Company (Monsanto), the assignee of the above-identified patent application;
3. That Dr. Needleman holds the positions of Senior Vice President of Research and Development at Searle and Chief

3. That Dr. Needleman holds the positions of Senior Vice President of Research and Development at Searle and Chief Scientist at Monsanto, whereas Dr. Glenn holds the titles of Fellow and Group Leader of Atherosclerosis Research at Searle;

4. That Elaine Krul, Ph.D., who is presently employed by the Nutrition and Consumer Sector, a division of the Monsanto Company, was formerly employed by Searle between February of 1994 and February of 1998 doing laboratory research related to atherosclerosis;

5. That during her employment by Searle, she carried out her research under the direction and control of one or both of Drs. Needleman and Glenn;

6. That the work described in this Declaration was carried out by Dr. Krul or a person under her direction and control, and was carried out in the State of Missouri, the United States of America;

7. That the work described in this Declaration was completed on a date prior to November 7, 1996, the date of publication of WO 996/34888;

8. That the documents attached to this Declaration are true copies of one or more of Dr. Krul's laboratory

notebooks and an invoice, except that the dates and notebook numbers have been obscured for the purposes of this Declaration;

9. That collective Exhibit 1 contains pages 158, 159 and 160 from one of her notebooks (notebook A) that contains the amino acid residue sequence of cholesteryl ester transfer protein (CETP), using single letter code, in which peptide sequences corresponding to RABCTP-2, RABCTP-3, RABCTP-4, RABCTP-5, RABCTP-6, and RABCTP-7 are underlined and identified as such;

10. That those peptides of Paragraph 9 correspond to SEQ ID Nos: 2, 3, 4, 5, 6, and 7, respectively of the above-identified application;

11. That enclosed Exhibit 2 is a copy of page 161 of that same notebook that reiterates the names of those polypeptides and provides a schematic of a branched oligolysine antigenic carrier molecule referred to a Multiple Antigenic Peptide Backbone that when conjugated to a polypeptide is also referred to as MAP in her notebooks, and is noted at page 18, lines 11-19 of the above-identified application;

12. That enclosed Exhibit 3 is a copy of an invoice from Genosys Biotechnologies, Inc., of The Woodlands, Texas (Genosys) directed to Dr. Krul for conjugates of MAP-covalently-bonded polypeptides RABCTP-2, -3, -4, -5, -6 and -7;

13. That twelve rabbits were immunized on two occasions, two each, with a MAP-linked polypeptide conjugate received from Genosys;

14. That those two immunizations were recorded on pages 165, 166 and 167 of her above notebook, copies of which are enclosed as collective Exhibit 4;

15. That the samples of the immunized rabbits' blood were taken thereafter and were assayed for total cholesterol (TC) and high density lipoprotein (HDL-C);

16. That enclosed Exhibit 5 is a copy of page .071 of a second of her notebooks (notebook B) that contains graphs of the total cholesterol (TC) and high density lipoprotein (HDL-C) for each of the rabbits used in the study from prior to the immunizations (day zero) through at least 75 days thereafter, and in which the boxes within each graph identify each peptide with a shortened designation (CTP-number rather than RABCTP-number) because of space considerations;

17. That those data indicate a general lowering of both total cholesterol and high density lipoprotein for each of the peptides used;

18. That total cholesterol was determined in a standard laboratory assay commercially available from Wako Pure

Chemical Industries, Inc. adapted to a 96-well microtiter plate format;

19. That high density lipoprotein was determined in a standard laboratory assay using a commercially available kit from Sigma Chemicals, Inc., of St. Louis, MO;

20. That enclosed Exhibit 6 is a copy of page 072 of her notebook B showing data for rabbits immunized with conjugates of free polypeptides RABCTP-2, -3, -4, -6 and -7 (again referred to as CTP-2, CTP-3 etc.), also obtained from Genosys, that were individually covalently bonded to tuberculin purified protein derivative (PPD) to form conjugates, and prepared as discussed in Example 1B of the above-identified application, beginning at page 37;

21. That the results shown in Exhibit 6 were obtained following two immunizations with the conjugates using the assay procedures discussed in relation to Exhibit 5;

22. That all statements made herein of his (their) own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the

United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Enclosures

Exhibits 1-6

2/1/99

Date


Philip Needleman

6 - January, 1999

Date


Kevin Glenn

Number

Subject

Project Number

160

Rabbit anti-CETP Project
Choice of Peptides for Immunization

SEARLE

RABBIT CETP SEQUENCE - RABCTP-5 / RABCTP-6 / RABCTP-7

RABCTP-7

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGY PDVSGERAVM

10	20	30	40	50
----	----	----	----	----

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

60	70	80	90	100
----	----	----	----	-----

YTSAWGLGINQSVDFEIDSAIDLQINTELTCDAGSVRTNAPDCYLAFHKL

110	120	130	140	150
-----	-----	-----	-----	-----

RABCTP-6

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

160	170	180	190	200
-----	-----	-----	-----	-----

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

210	220	230	240	250
-----	-----	-----	-----	-----

PPGLLGDSRMLYFWFSDQVLNSLARAQEQEGRLVLSLTGDEFKKVLETQG

260	270	280	290	300
-----	-----	-----	-----	-----

FDTNQEIFQELSRLGLPTGQAQVAVHCLKVPKISCQNRRGVVVSSSVAVTFR

310	320	330	340	350
-----	-----	-----	-----	-----

RABCTP-5

FPRPDGREAVAYRFEEDIITTVQASYSQKKFLHLILDFOCVPASGRAGSS

360	370	380	390	400
-----	-----	-----	-----	-----

ANLSVALRTEAKAVSNLTESRSSESLOSSLRLSLIATVGIPVMSRLEVAFT

410	420	430	440	450
-----	-----	-----	-----	-----

ALMNSKGDLFELIINPEIITLDGCLLQMDFGFPKHLLVDFLQLSLS

460	470	480	490
-----	-----	-----	-----

Signature

John Voss

D--

Read and Understood By

X / m

Date

Project Number

Subject

SEARLE

*Rabbit Anti-CETP Project
Choice of Peptides for Immunization*

Book Number

Page

159

RABBIT CETP SEQUENCE - RABCTP-3 / RABCTP-4

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGYPDVSGERAVM

| | | | |
10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

| | | | |
60 70 80 90 100

YTSAWGLGINQSVDFFEIDSAILQINTELTCDAGSVRTNAPDCYLAFHKL

| | | | |
110 120 130 140 150

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

| | | | |
160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTKHNVSEAFPLRAF

| | | | |
210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARAQFEGRLVLSLTGDEFKKVLETQG

| | | | |
260 270 280 290 300
RABCTP-3

FDTNQEIFQELSRLGLPTGQAQVAVHCLKVPKISCQNRGVVVSSSVAVTFR

| | | | |
310 320 330 340 350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS

| | | | |
360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSESLOSSLRSILATVGIPPEVMSRLEVAFT

| | | | |
410 420 430 440 450

ALMNSKGDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDFQSLIS

| | | | |
460 470 480 490

RABCTP-4

RABBIT CETP SEQUENCE - RABCTP-2

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGYPDVSGERAVM

| 10 | 20 | 30 | 40 | 50 |

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVFKGTLNYS

| 60 | 70 | 80 | 90 | 100 |

YTSAWGLGINQSVDFEIDS AIDI QINTELTCDAGSVRTNAPDCYLA FHKL

| 110 | 120 | 130 | 140 | 150 |

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

| 160 | 170 | 180 | 190 | 200 |

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

| 210 | 220 | 230 | 240 | 250 |

PPGLLGDSRMLYFWFSDQVLNSLARA AFQEGRLVLSLTGDEFKKVLETQG

| 260 | 270 | 280 | 290 | 300 |

FDTNQE~~I~~FOELSRLPTGQAQVAVHCLKVPKISCQNRGVVSSSVAVTFR

| 310 | 320 | 330 | 340 | 350 |

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS

| 360 | 370 | 380 | 390 | 400 |

ANLSVALRTEAKAVSNLTESRSES LQSSLRS LIATVG IPEVMSRLEV AFT

| 410 | 420 | 430 | 440 | 450 |

ALMNSKGDLF EIINPEI ITLDGCLLQMDFGF PKHLLVDFLQSL S

| 460 | 470 | 480 | 490 |

Jane Kruel

Read and Understood By
S. D. May

Project Number SEARLE	Subject Rabbit anti-CTP Project Choice of Peptides for Immunization	Book Number Page 161
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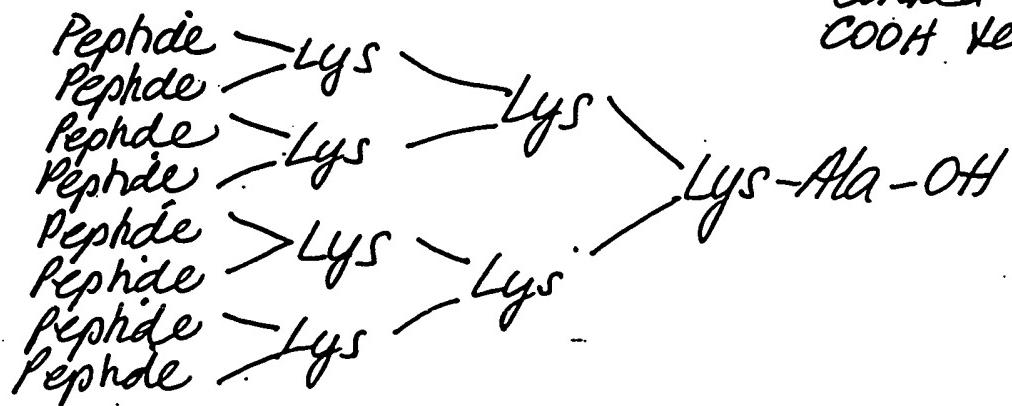
Summary of Peptide Sequences Submitted to Genesys
to couple on MAP:

RABCTP-2	306 - 325	(#001)	Order # 054215 P.O.# 8002455
RABCTP-3	345 - 364	(#002)	40µM of each
RABCTP-4	475 - 496	(#003)	Made Immuno Grade (>60% pure)
RABCTP-5	370 - 389	(#004)	Made on MAPS resin: see reference:
RABCTP-6	150 - 169	(#005)	Butz, S. et al. (1994) Peptide Research 7: 20-23.
RABCTP-7	42 - 61	(#006)	

Decided to try coupling to Multiple Antigenic Peptide Backbone, as a way to avoid "carrier" protein which will generate a lot of antibodies of and in itself.
One of the original references to this method is:

Posnett, DN, ~~McGrath~~ McGrath H, & Tam JP (1988)
J. Biol. Chem. 263: 1719-1725.

Structure of MAP:



NOTE: Peptides are linked via their COOH termini!!

Author's Signature

Elaine Krule

Read and Understood By

J. Nam

GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Order No.

Page 2

ORDER

54215

Bill To
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

ST. LOUIS MO 63167

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.
ST. LOUIS MO 63167

--CONTACT--

BARB GRIFFARD
314-694-1000 X 6825

--CONTACT--

314-694-4218

Customer: 100 6316701 KRU1
Salesman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
P/O Number: 8002455

ORDERED:

SHIPPED:

INVOICED:

LINE	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
5.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-6 LLL HLG GER EPG NLK QLF TH RESIDUES IMMUNO GRADE MAPS RESIN	~96 mg	20.00	35.00	950.00	MW 2393
6.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-7 DVS GER AVH LLG RVK YGL HH RESIDUES IMMUNO GRADE MAPS RESIN	~89 mg	20.00	35.00	950.00	MW 2215
Lot P157A-030 Peptide 54215-005									
Lot P157A-032 Peptide 54215-006									

COMMENTS:
cleared by 95% TFA, ether extracted 3X, suspended in SALES
[& 10% TFA/H₂O] - 1-2% acetic acid removed MISCELLANEOUS
+ lyophilized FREIGHT
↓ 4.624.00 TAX

TOT

PACKING LIST

GENOSYS 1-800-234-5200

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Stacey ext #127
Hogue

615-343-1465
James Tam

0.37 mM/g

0.37 μM/mg resin

Order No.

Dr. Stanfield

713-363-3693 ext 13:

Page

O R D E R

54215

Bill To
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

ST. LOUIS MO 63167

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.
ST. LOUIS MO 63167

--CONTACT--

BARB GRIFFARD
314-694-1000 X 6825

--CONTACT--

314-694-4218

Customer: 100 6316701 KRUI

ORDERED:

Salesman: 8

SHIPPED:

Terms: NET 30

INVOICED:

SHIP VIA: Airborne (Prepaid)

/O Number: 8002455

LINE	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
1.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-2 EIF QEL SRG LPT GQA QVA VH RESIDUES IMMUNO GRADE MAPS RESIN	~87 mg	20.00	35	950	
			MW 2181						
Lot P157A-022									
peptide # 54215-001									
2.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-3 YAV TFR FPR PDG REA YAV RF RESIDUES IMMUNO GRADE MAPS RESIN	~94 mg	20.00	35	950	
			MW 2355						
Lot P157A-024									
peptide # 54215-002									
3.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-4 LLL QMD FGF PKH LLV QFL QSL S RESIDUES IMMUNO GRADE MAPS RESIN	~102 mg	22.00	35	950	
			MW 2562						
Lot P157A-026									
peptide # 54215-003									
4.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-5 TTV QAS YSO KKL FLH LLD FO RESIDUES IMMUNO GRADE MAPS RESIN	~95 mg	20.00	35	950	
			MW 2369						
Lot P157A-028									
peptide # 54215-004									

ACKING LIST

Project Number:	Subject	Rabbit anti-CEPP Project Immunization Procedure	Book Number
SEARLE			Page 165

Immunization of Rabbit

Subcutaneously administered (0.05-0.1 ml/site)
along rabbit's back - done by Margi Baldwin.

Rabbits in Room 105 (Tattooed)

- | | | |
|----------|----------|----------------------------|
| #1, #2 | RABCTP-2 | (3.3 mg total ÷ 2 rabbits) |
| #3, #4 | RABCTP-3 | (3.4 mg ") |
| #5, #6 | RABCTP-4 | (3.2 mg ") |
| #7, #8 | RABCTP-5 | (3.2 mg ") |
| #9, #10 | RABCTP-6 | (3.3 mg ") |
| #11, #12 | RABCTP-7 | (3.5 mg ") |

INVESTIGATOR: _____ DATE: _____
 room #: _____ phone #: _____ Mail Zone: TAm
 Technician: _____

RE-
WELCH
ERA
Heated

Animal ID #	Bleed	Serum/Plasma	Other	Initials
1	10	—	—	H
2	10	—	—	Q
3	10	—	—	JH
4	10	—	—	Q
5	10	—	—	Q
6	10	—	—	JH
7	10	—	—	Q
8	10	—	—	JH
9	10	—	—	Q
10	10	—	—	H
11	10	—	—	Q
12	10	—	—	H

INVESTIGATOR: KruelROOM NO. 105

DATE: _____

TECH: JT + CMSPECIES: Rabbit

ANIMAL ID #	WEIGHT (LBS/KG) S	OTHER
1	1900	
2	1901.1	
3	2070.1	
4	2051.7	
5	2154.3	
6	2145.0	
7	2040	
8	2116	
9	2002.8	
10	1942	
11	1945.4	
12	2084	

Rabbit's Initial Weights

Second Immunization -

On weighed out the following: (whole resin)

RABCTP-2	3.5 mg	(^{into} 2 rabbits)
RABCTP-3	4.3	"
" - 4	4.7	"
" - 5	4.0	"
" - 6	3.8	"
" - 7	4.2	"

Dissolved in 1.5 ml sterile PBS, pH 7.4.

According to Butz, S. et al. (1994) Peptide Research 7: 20-23 sonication of beads leads to partial breakage of bead to make it more accessible to immune system.

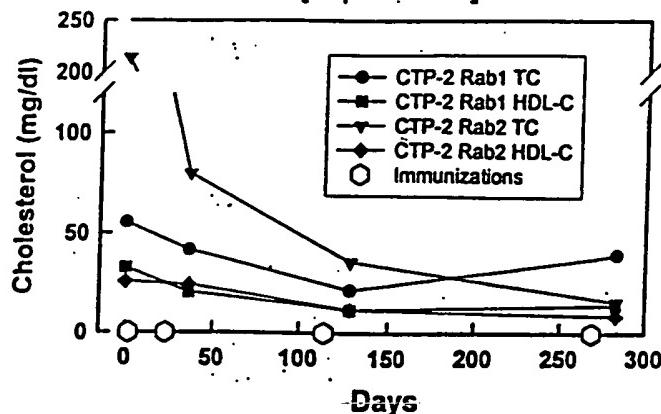
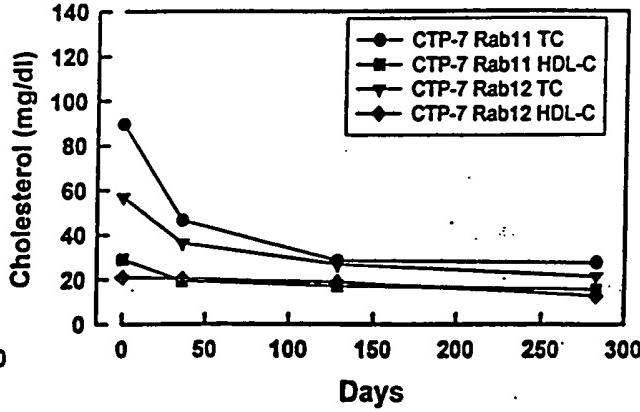
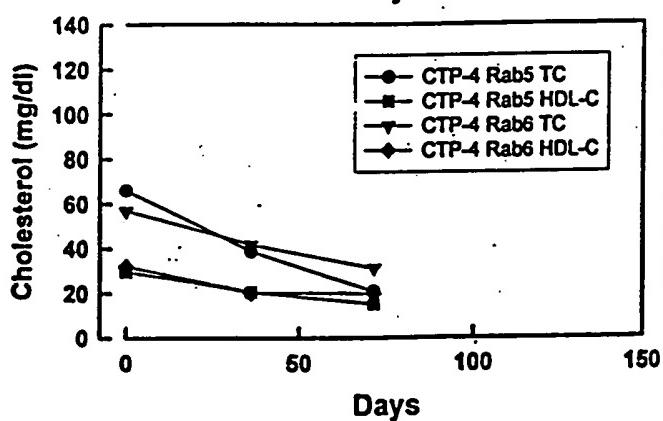
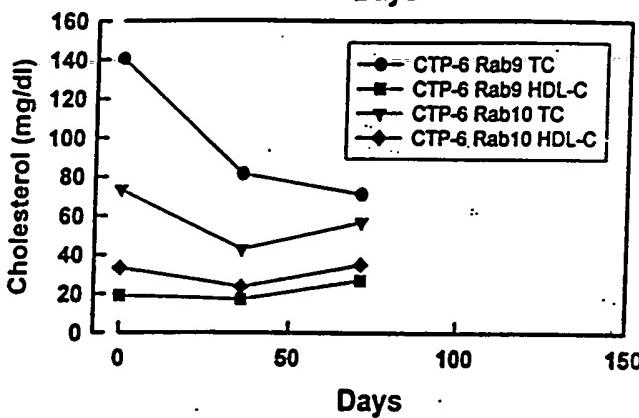
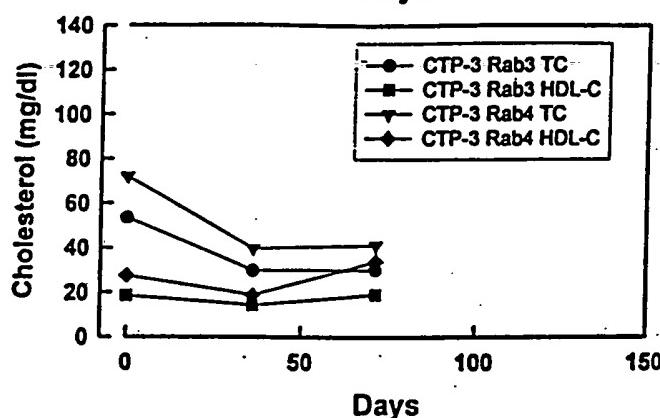
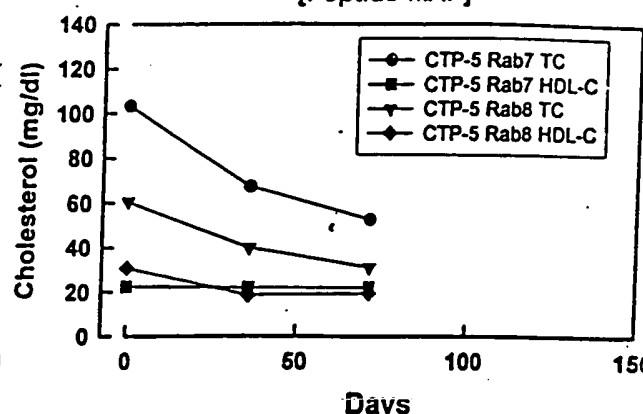
Solutions of beads were sonicated with microtip at maximum setting for 2-3 minutes (min, num). Not such change in turbidity of solution - beads still look intact & settle out quickly. Continued to indicate - each up to 5 minutes with little change. (It is not clear how long the Butz paper authors sonicated their beads).

Left beads in PBS @ 4°C overnight.

In warmed bead solutions to room temperature. Added 1.5 ml INCOMPLETE Freund's Adjuvant (Sigma). Emulsified using 2 x 5cc syringes as before (p. 165).

Immunized rabbit subcutaneously (~1.5 ml of each emulsion) in multiple sites on back.

Records to be taken on:

Project Number
SEARLESubject
*Summary of Lipid R� wps on
Antibera from CTP-Pepside
Immunized Rabbit*Book Number
www
Page**071**Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]

Conclusions: Initial drop in cholesterol may be adaptation to new diet (Vendor vs. Searle) or consequence of immunizing per se. Possible effect of CTP-3, CTP-6 immunizations in raising HDL. Curiously no Ab bound to peptide on ELISAs (see 5634011) for

Author's Signature

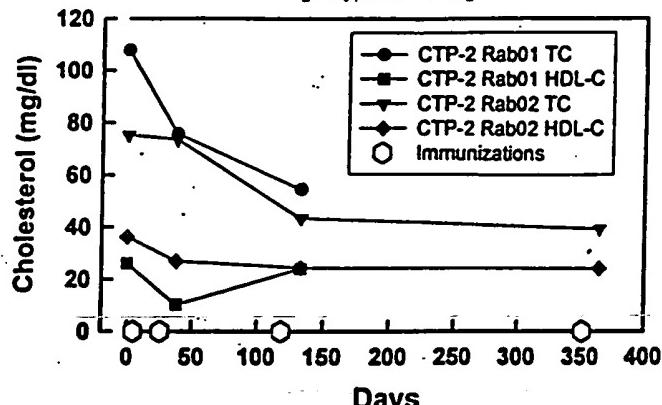
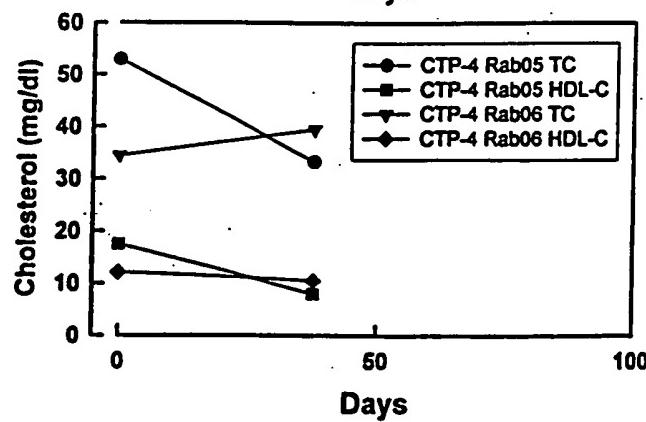
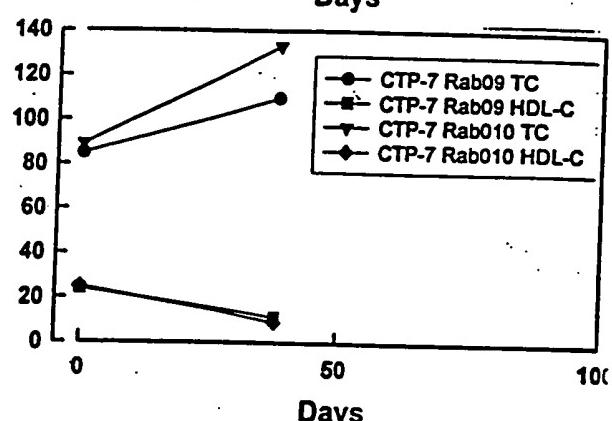
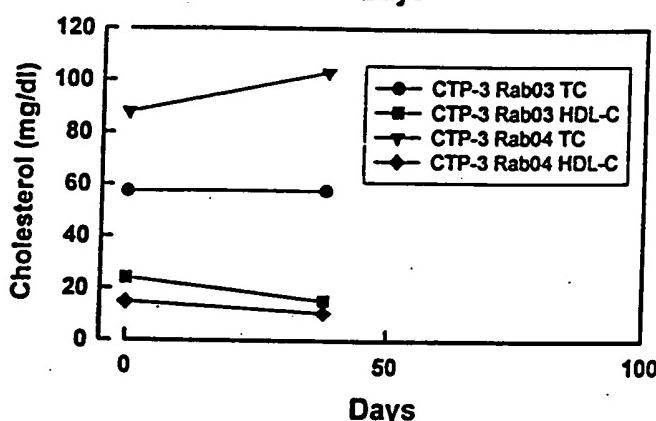
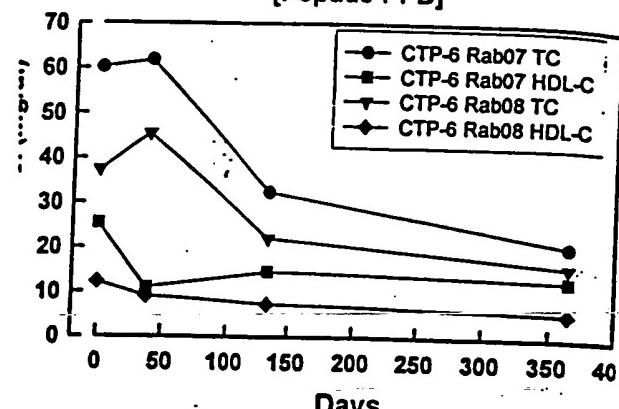
Elaine Kurl

Date

Read and Understood By

Denise Nachmanek

D

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]

*ELISA results
See

Conclusion: Drop in cholesterol seen over time. Not clear why. Only CTP-2 & CTP-6 showed Abs to peptide on ELISA. One rabbit that died (CTP-2) appeared to have HDL raising. Also rabbit 07 (CTP-6) appears to have HDL elevation. No effect on HDL seen in CTP-3, CTP-4 or CTP-7.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Needleman et al.)
Serial No.: 08/788,882) Attorney Docket
Filed: January 21, 1997) 6221/69242
For: AN IMMUNOLOGICAL PROCESS AND)
CONSTRUCTS FOR INCREASING) MON-102.0
THE HDL CHOLESTEROL CONCENTRATION)
Examiner: T. Scheiner)
)

DECLARATION OF PHILIP NEEDLEMAN AND
KEVIN GLENN PURSUANT TO 37 C.F.R. §1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PHILIP NEEDLEMAN, Ph.D. and KEVIN GLENN, Ph.D.

Declare:

1. That they are the Philip Needleman and Kevin Glenn
that are the named inventors of the subject patent application;
2. That they are employed by G.D. Searle, Co.,
(Searle) a wholly owned subsidiary of the Monsanto Company
(Monsanto), the assignee of the above-identified patent
application;
3. That Dr. Needleman holds the positions of Senior
Vice President of Research and Development at Searle and Chief

Considered
15
9/10/99

Scientist at Monsanto, whereas Dr. Glenn holds the titles of Fellow and Group Leader of Atherosclerosis Research at Searle;

4. That Elaine Krul, Ph.D., who is presently employed by the Nutrition and Consumer Sector, a division of the Monsanto Company, was formerly employed by Searle between February of 1994 and February of 1998 doing laboratory research related to atherosclerosis;

5. That during her employment by Searle, she carried out her research under the direction and control of one or both of Drs. Needleman and Glenn;

6. That the work described in this Declaration was carried out by Dr. Krul or a person under her direction and control, and was carried out in the State of Missouri, the United States of America;

7. That the work described in this Declaration was completed on a date prior to November 7, 1996, the date of publication of WO 996/34888;

8. That the documents attached to this Declaration are true copies of one or more of Dr. Krul's laboratory notebooks and an invoice, except that the dates and notebook numbers have been obscured for the purposes of this Declaration;

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10. That those peptides of Paragraph 9 correspond to SEQ ID Nos: 2, 3, 4, 5, 6, and 7, respectively of the above-identified application;

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14. That those two immunizations were recorded on pages 165, 166 and 167 of her above notebook, copies of which are enclosed as collective Exhibit 4;

15. That the samples of the immunized rabbits' blood were taken thereafter and were assayed for total cholesterol (TC) and high density lipoprotein (HDL-C);

16. That enclosed Exhibit 5 is a copy of page 071 of a second of her notebooks (notebook B) that contains graphs of the total cholesterol (TC) and high density lipoprotein (HDL-C) for each of the rabbits used in the study from prior to the immunizations (day zero) through at least 75 days thereafter, and in which the boxes within each graph identify each peptide with a shortened designation (CTP-number rather than RABCTP-number) because of space considerations;

17. That those data indicate a general lowering of both total cholesterol and high density lipoprotein for each of the peptides used;

18. That total cholesterol was determined in a standard laboratory assay commercially available from Wako Pure

Chemical Industries, Inc., adapted to a 96-well microtiter plate format;

19. That high density lipoprotein was determined in a standard laboratory assay using a commercially available kit from Sigma Chemicals, Inc., of St. Louis, MO;

20. That enclosed Exhibit 6 is a copy of page 072 of her notebook B showing data for rabbits immunized with conjugates of free polypeptides RABCTP-2, -3, -4, -6 and -7 (again referred to as CTP-2, CTP-3 etc.), also obtained from Genosys, that were individually covalently bonded to tuberculin purified protein derivative (PPD) to form conjugates, and prepared as discussed in Example 1B of the above-identified application, beginning at page 37;

21. That the results shown in Exhibit 6 were obtained following two immunizations with the conjugates using the assay procedures discussed in relation to Exhibit 5;

22. That all statements made herein of his (their) own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the

United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Enclosures

Exhibits 1-6

Date

2/1/99

Philip Needleman

Philip Needleman

Date

6 January, 1999

Kevin Glenn

Kevin Glenn

Number

Subject

Project Number

160

Rabbit anti-CETP Project
Choice of Peptides for Immunization

SEARLE

RABBIT CETP SEQUENCE - RABCTP-5 / RABCTP-6 / RABCTP-7

RABCTP-7

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGYPDVSGERAVM

| 10 | 20 | 30 | 40 | 50 |

LLGRVKYGLHNLQISHLSIASSQVELDAKTIDVAIQNVSVFKGTLNYS

| 60 | 70 | 80 | 90 | 100 |

YTSAWGLGINQSVD~~FEIDS~~AIDLQINTELCDAGSVRTNAPPDCYLAFHKL

| 110 | 120 | 130 | 140 | 150 |

RABCTP-6

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

| 160 | 170 | 180 | 190 | 200 |

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTKKNVSEAFPLRAF

| 210 | 220 | 230 | 240 | 250 |

PPGLLGDSRMLYFWFSDQVLNSLARA~~AFQEGR~~LVL~~S~~TGDEFKKVLETQG

| 260 | 270 | 280 | 290 | 300 |

FDTNQE~~IFQ~~ELSRGLPTGQAQAVAHCLKVPKISCQNREGVVVSSSVAVTFR

| 310 | 320 | 330 | 340 | 350 |

RABCTP-5

FPRPDGREAVAYRFEEDI~~ITTVQASY~~SQKKFLHLLDFOCVPASGRAGSS

| 360 | 370 | 380 | 390 | 400 |

ANLSVALRTEAKAVSNLTESRSES~~LQSSL~~RIATVG~~IPEV~~MSRLEVAFT

| 410 | 420 | 430 | 440 | 450 |

ALMNSKG~~LDL~~FEIINPEI~~ITLDG~~CLLQMDFGFPK~~HLL~~VDFLQSL~~S~~

| 460 | 470 | 480 | 490 |

Project Number

Subject

SEARLE

Rabbit Anti-CETP Project
Choice of Peptides for Immunization

Book Number

Page

159

RABBIT CETP SEQUENCE - RABCTP-3 / RABCTP-4.

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGYPDVSGERAVM

| 10 | 20 | 30 | 40 | 50 |

LLGRVKYGLHNLQISHLSIASSQVELDAKTIDVAIQNVSVVFKGTLNYS

| 60 | 70 | 80 | 90 | 100 |

YTSAWGLGINQSVDFEIDSAYDLQINTELTCDAGSVRTNAPDCYLAFHKL

| 110 | 120 | 130 | 140 | 150 |

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

| 160 | 170 | 180 | 190 | 200 |

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

| 210 | 220 | 230 | 240 | 250 |

PPGLLGDSRMLYFWFSDQVLNSLARAQEGRLVLSLTGDEFKKVLETQG

| 260 | 270 | 280 | 290 | 300 |

RABCTP-3

FDTNQEIFQELSRLPTGQAQVAVHCLKVPKISCQNRGVVVSSSVAVTFR

| 310 | 320 | 330 | 340 | 350 |

FPRPDGREAVAYRFEEDIITTVQASYSQKKFLHLLDFQCVPASGRAGSS

| 360 | 370 | 380 | 390 | 400 |

ANLSVALRTEAKAVSNLTESRSESLOSSLRSLIATVGIPPEVMSRLEVAFT

| 410 | 420 | 430 | 440 | 450 |

ALMNSKGDLFEIINPEIITLDGCLLLQMDFGFPKHLLVDLQLSLS

| 460 | 470 | 480 | 490 |

RABCTP-4

RABBIT CETP SEQUENCE - RABCTP-2

CPKGASYEAGIVCRITKPALLVLNQETAKVVQTAFQRAGYPDVSGERAVM

| | | | |
10 20 30 40 50

LLGRVKYGLHNLQISHLSIASSQVELVDAKTIDVAIQNVSVVFKGTLNYS

| | | | |
60 70 80 90 100

YTSAWGLGINOSVDFEIDSAYDLOINTELTCAGSVRTNAPDCYLAFHKL

| | | | |
110 120 130 140 150

LLHLQGEREPGWLKQLFTNFISFTLKLILKGQVCNEINTISNIMADFVQT

| | | | |
160 170 180 190 200

RAASILSDGDIGVDISVTGAPVITATYLESHHKGHFTHKNVSEAFPLRAF

| | | | |
210 220 230 240 250

PPGLLGDSRMLYFWFSDQVLNSLARAQEQEGRLVLSLTGDEFKKVLETQG

| | | | |
260 270 280 290 300FDTNQEIFQELSRLPTGQAQVAVHCLKVPKISCQNRGVVVSSSVAVTFR| | | | |
310 320 330 340 350

FPRPDGREAVAYRFEEDIITTVQASYSQKKLFLHLLDFQCVPASGRAGSS

| | | | |
360 370 380 390 400

ANLSVALRTEAKAVSNLTESRSESLOSSLRSLIATVGIPPEVMSRLEVAFT

| | | | |
410 420 430 440 450

ALMNSKGDLFEEIINPEIITLDGCLLQLMDFGFPKHLLVDFLQSLS

| | | | |
460 470 480 490

Anne Kruel

Read and Understood By
S. May

D--

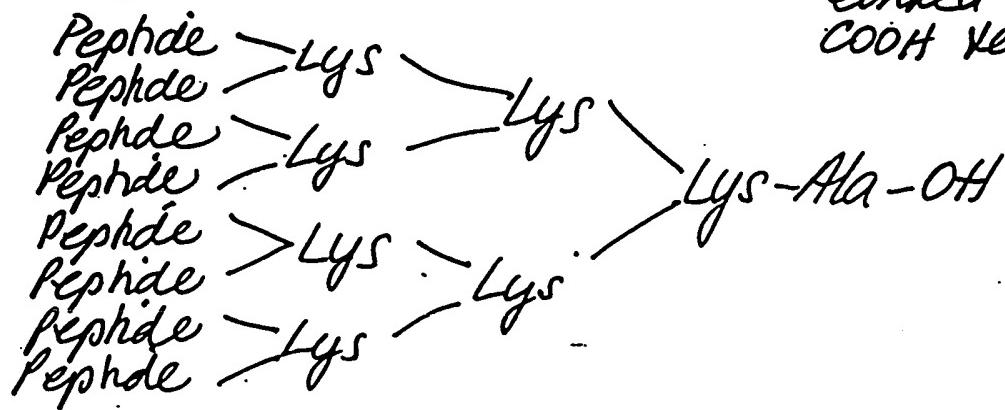
Summary of Peptide Sequences Submitted to Genasys
to couple on MAP:

RABCTP-2	306 - 325	(#001)	Order # 054215 P.O.# 8002455
RABCTP-3	345 - 364	(#002)	40µM of each
RABCTP-4	475 - 496	(#003)	Made Immuno Grade (>60% pure)
RABCTP-5	370 - 389	(#004)	Made on MAPS resin:
RABCTP-6	150 - 169	(#005)	see reference: Butz, S. et al. (1994) Peptide Research 7: 20-23.
RABCTP-7	42 - 61	(#006)	

Decided to try coupling to Multiple Antigenic Peptide Backbone, do a way to avoid "carrier" protein which will generate a lot of antibodies of and in itself.
One of the original references to this method is:

Posnett, D.N., ~~McGrath~~ McGrath H., & Tam JP (1988).
J. Biol. Chem. 263: 1719-1725.

Structure of MAP:



NOTE: Peptides are linked via their COOH termini!!

GENOSYS 1-800-234-2127

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Stacey
Hogue

615-343-1465
James Tam

0.37 mm/g

0.37 μM/mg Resin

Order No.

Dr. Stanfield

Page
713-363-3693 ext 13:

O R D E R

54215

Bill To
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

ST. LOUIS MO 63167

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.
ST. LOUIS MO 63167

—CONTACT—

BARB GRIFFARD
314-694-1000 X 6825

—CONTACT—

314-694-4216

Customer: 100 6316701 KRU1
Sal sman: 8
Terms: NET 30
SHIP VIA: Airborne (Prepaid)
/O Number: B002455

ORDERED

SHIPPED

INVOICED

LINE	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC.	NET PRICE	EXTENSION
1.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-2					
		MW 2181		EIF QEL SRG LPT GQA QVA VH RESIDUES	20.00	35.00			
		Lot P157A-022		IMMUNO GRADE					
		peptide # 54215-001		NAPS RESIN	~87 mg				
2.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-3					
		Lot P157A-024		VAV TFR FPR PDG REA VAY RF RESIDUES	20.00	35.00			
		MW 2355		IMMUNO GRADE					
		peptide # 54215-002		NAPS RESIN	~94 mg				
3.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-4					
		Lot # P157A-026		LLL QMD FGF PKH LLV OFL QSL S RESIDUES	22.00	35.00			
		MW 2562		IMMUNO GRADE					
		peptide # 54215-003		NAPS RESIN	~102 mg				
4.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 uM IMMUNO GRADE RABCTP-5					
		MW 2369		TTV QAS YSQ KKL FLH LLC FQ RESIDUES	20.00	35.00			
		Lot P157A-028		IMMUNO GRADE					
		peptide # 54215-004		NAPS RESIN	~95 mg				

ACKING LIST

GENOSYS

Genosys Biotechnologies, Inc.
1442 Lake Front Circle, Suite 185
The Woodlands, TX 77380-3600
(713) 363-3693
FAX (713) 363-2212

Order No.

Page 2

ORDER

S4215

Bill T
MONSANTO COMPANY
ACCOUNTS PAYABLE, N2F
800 NORTH LINDBERGH BLVD.

ST. LOUIS MO 63167

Ship To
DR. ELAINE KRULL
MONSANTO COMPANY
60-000-760.92
T213W/T2M
800 N. LINDBERGH BLVD.

ST. LOUIS MO 63167

CONTACT

BARB GRIFFARD
314-694-1000 X 6825

CONTACT

314-694-4218

Customer: 100 6316701 KRU1

ORDERED:

Salesman: 8

SHIPPED:

Terms: NET 30

INVOICED:

SHIP VIA: Airborne (Prepaid)

P/O Number: B002455

ITEM	ORDERED	SHIPPED	PRODUCT	DESCRIPTION	QUANTITY	UNIT PRICE	DISC	NET PRICE	EXTENSION
5.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-6					
				LLL HLG GER EPG NLK QLF TH RESIDUES		20.60			
				IMMUNO GRADE MAPS RESIN					
MW 2393									
+ P157A-030									
rephde 54215-005									
6.000	1.00	0.00	PEPIG/40	CUSTOM PEPTIDE 40 UM IMMUNO GRADE RABCTP-7					
				DVS GER AVK LLG RVK YGL HH RESIDUES		20.00			
				IMMUNO GRADE MAPS RESIN					
MW 2215									
lot P157A-032									
rephde 54215-006									

COMMENTS:

cleared by 95% TFA, ether extracted 3X, suspended in SALE
8 Molar H2O — 1-2% acetic acid resuspended MISCELLANEOUS
+ lyophilized FREIGHT
↓ 4.624.00 TAX
lyophilized TOT

PACKING LIST

Project Number:	Subject:	Rabbit anti-CETP Project Immunization Procedure	Book Number:
SEARLE			Page 165

Immunization of Rabbits

Subcutaneously administered (0.05-0.1 ml/site)
along rabbit's back - done by Margi Baldwin.

Rabbits in Room 105 (Tattooed)

- | | | | |
|----------|----------|----------------------------|--|
| #1, #2 | RABCTP-2 | (3.3 mg total ÷ 2 rabbits) | |
| #3, #4 | RABCTP-3 | (3.4 mg ") | |
| #5, #6 | RABCTP-4 | (3.2 mg ") | |
| #7, #8 | RABCTP-5 | (3.2 mg ") | |
| #9, #10 | RABCTP-6 | (3.3 mg ") | |
| #11, #12 | RABCTP-7 | (3.5 mg ") | |

INVESTIGATOR: _____ DATE: _____
 room #: _____ phone #: _____ Mail Zone: TAm
 Technician: _____

RE-
vaccine
era
selected

red
seen in
liquor
-20°C.

Animal ID #	Bleed	Serum/Plasma	Other	Initials
1	10	—	—	H
2	10	—	—	Q
3	10	—	—	Q
4	10	—	—	H
5	10	—	—	Q
6	10	—	—	H
7	10	—	—	Q
8	10	—	—	H
9	10	—	—	Q
10	10	—	—	H
11	10	—	—	Q
12	10	—	—	H

INVESTIGATOR: Kruel

ROOM NO. 105

DATE:

TECH: JT + GM

SPECIES: Rabbit

ANIMAL I.D. #	WEIGHT (lbs/kg) S	OTHER
1	1900	
2	1901.1	
3	2070.1	
4	2051.7	
5	2154.3	
6	2145.10	
7	2040	
8	2116	
9	2002.8	
10	1942	
11	1965.4	
12	2084	

Rabbit's Initial Weights

Second Immunization -

On weighed out the following: (whole resin)

RABCTP-2	3.5 mg	(2 nd rabbit)
RABCTP-3	4.3	"
" - 4	4.7	"
" - 5	4.0	"
" - 6	3.8	"
" - 7	4.2	"

Dissolved in 1.5 ml sterile PBS, pH 7.4.

According to Butz, S. et al. (1994) Peptide Research 7: 20-23 sonication of beads leads to partial breakage of bead to make it more accessible to immune system.

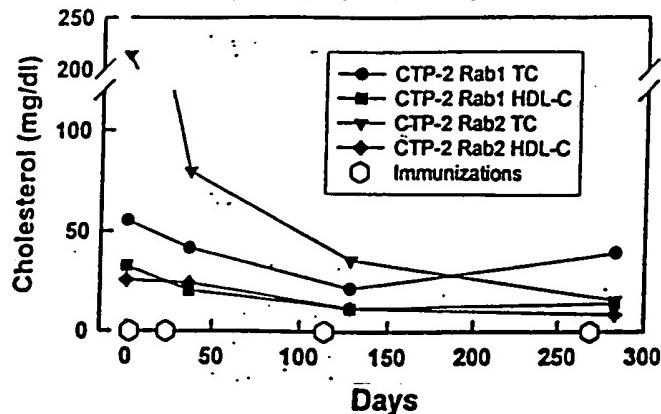
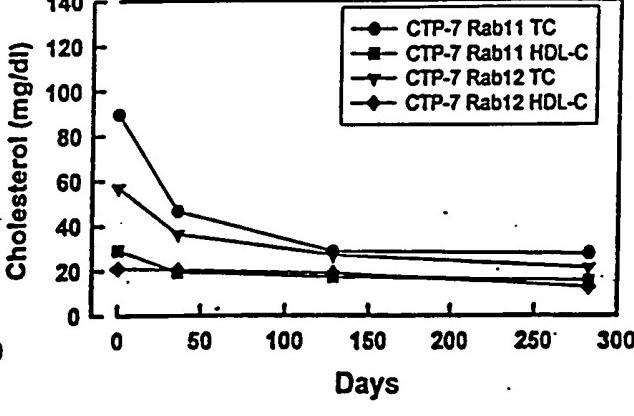
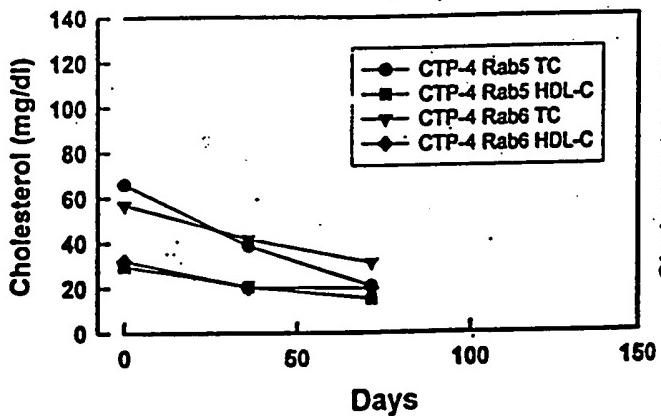
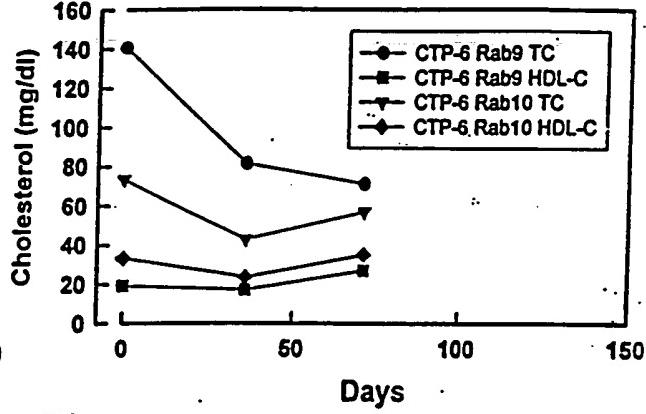
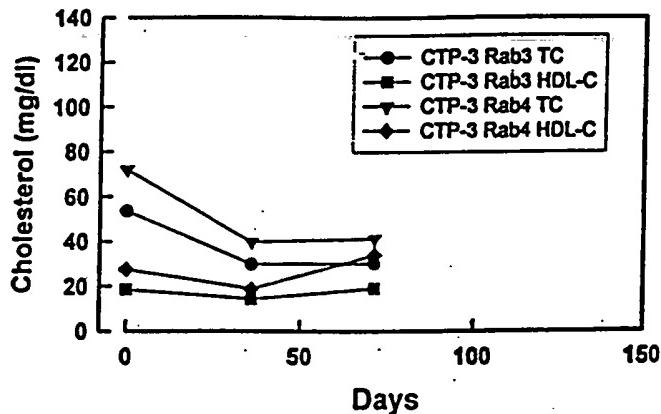
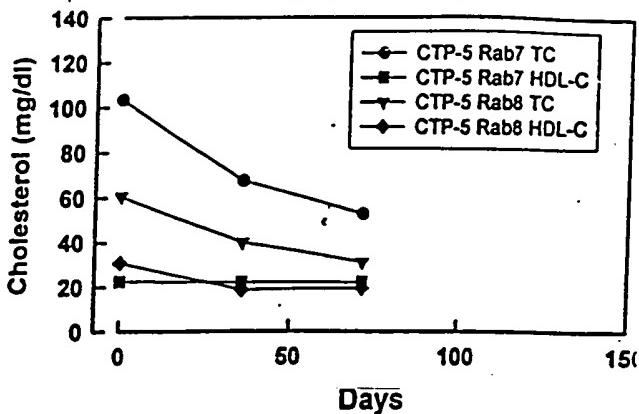
Solutions of beads were sonicated with microtip at maximum setting for 2-3 minutes (min, max). No such change in turbidity of solution - beads still look intact & settle out quickly. Continued to sonicate - each up to 5 minutes with little change. (It is not clear how long the Butz paper authors sonicated their beads).

Left beads in PBS @ 4°C overnight.

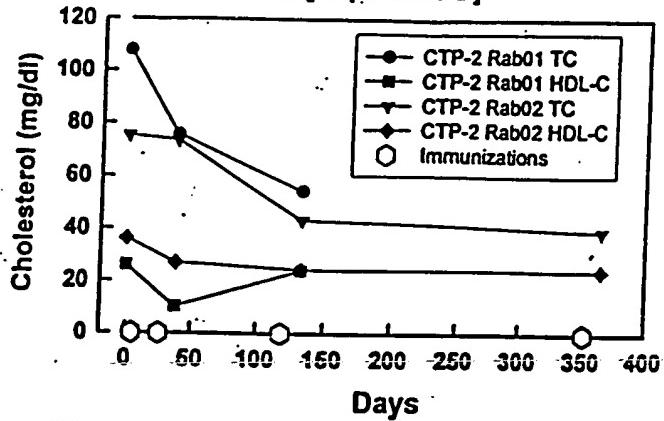
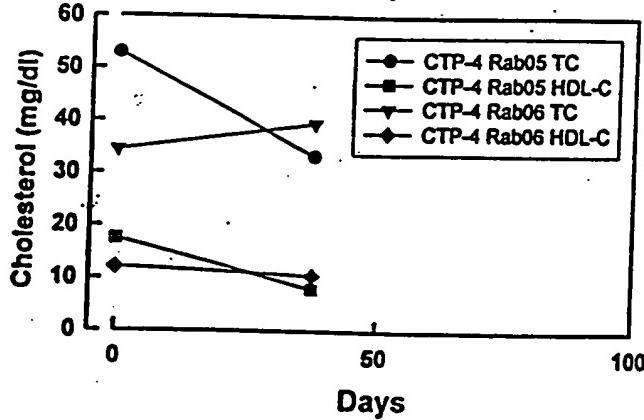
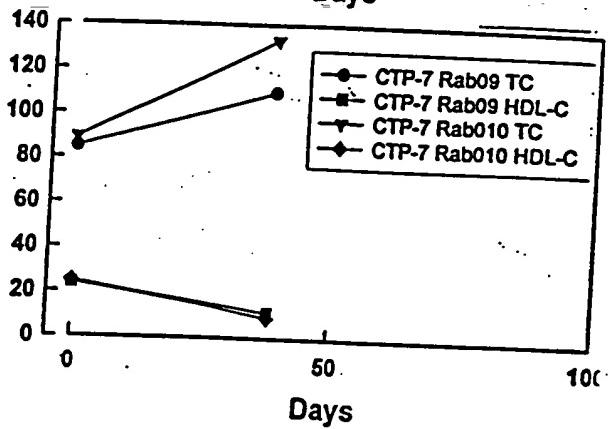
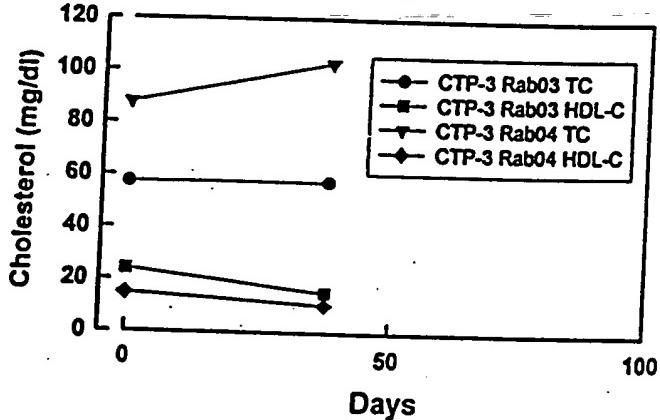
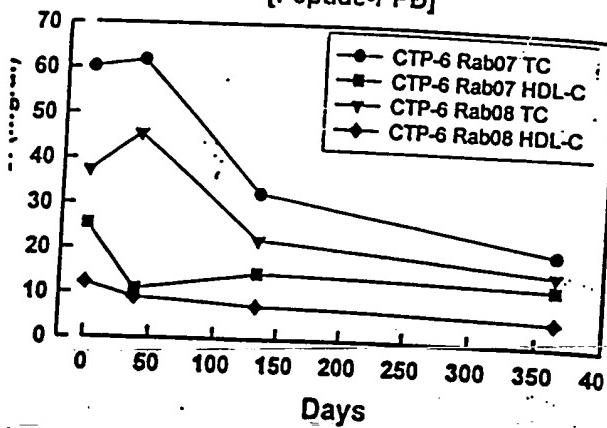
In warmed bead solution to room temperature. Added 1.5 ml INCOMPLETE Freund's Adjuvant (Sigma). Emulsified using 2 x 5cc syringes as before (p. 165).

Immunized rabbit subcutaneously (~1.5 ml of each emulsion) in multiple sites on back.

Records to be taken on:

**Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]****Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-MAP]**

Conclusions: Initial drop in cholesterol may be adaptation to new diet (Vendor vs. Searle) or consequence of immunizing per se. Possible effect of CTP-3, CTP-6 immunizations in raising HDL. Curiously no Ab bound to peptide on ELISAs (see 5634011).

Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]Total & HDL Cholesterol
in Immunized Rabbits
[Peptide-PPD]

*ELISA results
See

Conclusion: Drop in cholesterol seen over time. Not clear why only CTP-2 & CTP-6 showed Abs to peptide on ELISA. One rabbit that died (CTP-2) appeared to have HDL raising. Also rabbit 07 (CTP-6) appears to have HDL elevation. No effect on HDL seen in CTP-3, CTP-4 or CTP-7.

Author's Signature

Elaine Kurl

Date

Read and Understood By

Denise Nachonick

Date